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Short communication

A rapid and simple determination of protoberberine alkaloids in Rhizoma Coptidis by ^{1}H NMR and its application for quality control of commercial prescriptions

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ABSTRACT

Simple, convenient, sensitive and accurate analytical methods are needed for the analysis of alkaloid components in Rhizoma Coptidis in traditional Chinese herbal medicine, which has important bioactivity. In the present study, a highly specific and sensitive method using ^{1}H NMR has been developed for the quantitative determination of protoberberine alkaloids berberine, palmatine, coptisine and jatrorrhizine in *Coptis* species and their commercial traditional Chinese medicine prescriptions. A ^{1}H NMR analysis of the H-13 signals of target protoberberine alkaloids was performed. By comparing the solvent effects on the resolution of these signals, methanol-d_4–benzene-d_6 (75:25) is selected as an optimal ^{1}H NMR solvent. The quantity of the compounds is calculated by the relative ratio of the integral values of the target peak for each compound to the known amount of the internal standard anthracene. This method allows rapid and simple quantitation of protoberberine alkaloids from *Coptis* species and the more complex commercial prescriptions in 5 min without any pre-purification steps. The recoveries of these alkaloids from *Coptis chinensis* are in the range of 93–105%. Limit of detection of berberine in the plant material or prescription is 0.03 mg/mL. The advantages of this method are that no reference compounds are required for calibration curves, the quantification can be directly realized on a crude extract, and the better selectivity for protoberberine alkaloids and a very significant time-gain can be achieved, in comparison to conventional HPLC methods, for instance.

1. Introduction

Rhizoma Coptidis (Huanglian in Chinese) is considered to be one of the 50 fundamental herbs in Chinese herbalism, commonly used to treat a variety of diseases, such as headache, fever, gnostids, constipation and diarrhoea. Besides, Rhizoma Coptidis extracts and decoctions have demonstrated significant antimicrobial activity against a variety of organisms including bacteria, viruses, fungi, protozoans, helminthes, and chlamydia [1]. Protoberberine alkaloids (Fig. 1) berberine, palmatine, coptisine, jatrorrhizine and berberastine are reported to contribute for the biological activity of this species. The pharmacological actions of protoberberine alkaloids include metabolic inhibition of certain organisms, inhibition of smooth muscle contraction, reduction of inflammation, and stimulation of bile and bilirubin secretion. Currently, the predominant clinical uses of protoberberine alkaloids include bacterial diarrhoea, intestinal parasite infections, and ocular trachoma infections [2,3]. Although these alkaloids are not considered to be toxic at doses used in clinical situation, side effects including constipation, dyspnoea, lowered blood pressure, flu-like symptoms, and cardiac damage may result from taking high dosages. It should be avoided in pregnancy to take Berberine. Because of its bilirubin displacement properties [4], it caused uterine contractions and miscarriage, and in jaundiced neonates. Rhizoma Coptidis used in oriental medicine showed quite variable qualities, because of a number of species *Coptis* species comprise the source of Rhizoma Coptidis on the market. Moreover, the diversities in geographical cultivations make the content of active alkaloids quite different from each other. Thus, it is necessary to determine the species in Rhizoma Coptidis used in commercial prescriptions and the contents of protoberberine alkaloids in plant material or prescriptions.

The quantitative methods for the determination protoberberine alkaloids in *Coptis* species based on high-performance liquid chromatography (HPLC) and capillary electrophoresis (CE) have been described [5–9]. Controlling pH values of the mobile phase was needed to increase the resolution. Electrospray ionization tandem mass (ESI-MS) [10] and immunoquantitative analysis method [11] were also reported for the analysis of protoberberine alkaloids.

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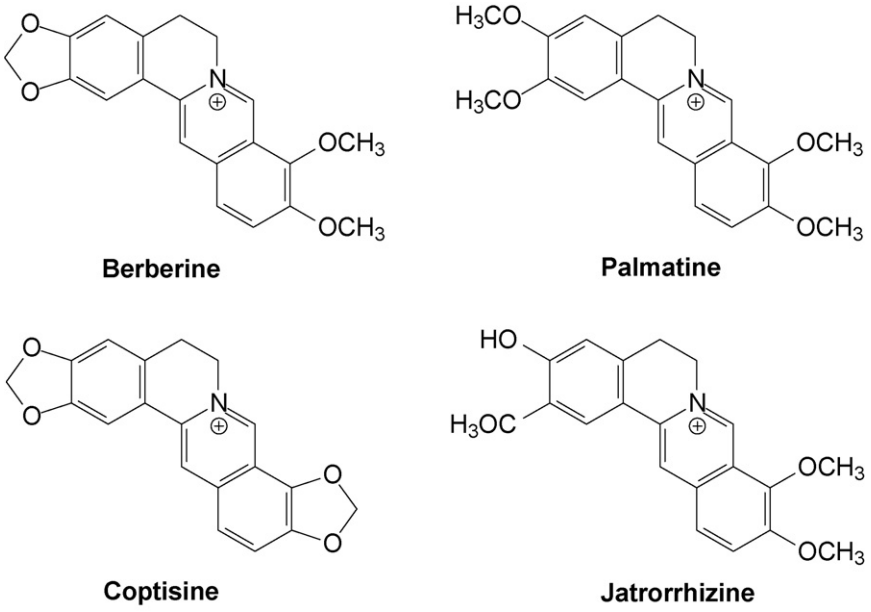

Fig. 1. Structures of the protoberberine alkaloids in Rhizoma Coptidis: berberine, palmatine, coptisine, and jatrorrhizine.

However, disadvantages such as low sensitivity, time-consuming, inextricable preparation steps, and the requirement for extensive derivatization steps remain problematic in the reported methods. For better quality control of these pharmaceutically important alkaloids in *Coptis* species and commercial prescriptions, it is highly desirable to discover the suitable method.

Recently, high-resolution nuclear magnetic resonance (NMR) spectroscopy is developing into an important tool in quality control of phyto-preparations [12–17] and in clinical diagnosis and monitoring of treatment [18]. The advantages of ^{1}H NMR method are manifold, viz. it is rapid, noninvasive and any sample pre-clean steps are not required. In addition, there is no standard compound needed for preparing the calibration curves and it detects all the components present in herbal preparations simultaneously in a single measurement. Therefore, we hypothesized that NMR spectroscopy might be superior to the conventional HPLC for the analysis of protoberberine alkaloids. In this paper, we describe the quantitative analysis of protoberberine alkaloids berberine, palmatine, coptisine and jatrorrhizine from *Coptis* species and their commercial traditional Chinese medicine prescriptions (23 prescriptions from four pharmaceutical companies) using ^{1}H NMR spectroscopy. This method allows rapid and simultaneous determination of the four protoberberine analogues without any pre-cleaning steps.

2. Experimental

2.1. Chemicals

First grade methanol and anthracene are purchased from E. Merck (Darmstadt, Germany). Methanol-d_4 (99.9%) and benzene-d_6 (99.6%) are obtained from Aldrich (Milwaukee, WI, USA). The reference compounds (berberine and palmatine) were isolated from the roots of *P. amurense* in a prior study [19]. The purity of reference compounds is checked by NMR and HPLC methods (>99.7%).

2.2. Materials

The dried bark of *Coptis chinensis*, *C. teeta* and *C. deltoidea* was collected from Bureau of Food and Drug Analysis, Department of Health, Executive Yuan, Taiwan, R.O.C. in August 2004 and verified by Prof. C.S. Kuoh. The commercial traditional Chinese medicine prescriptions were purchased from Kaiser Pharmaceutical Company (Tainan, Taiwan), Chuang Song Zong Pharmaceutical Company (Kaohsiung, Taiwan), Sun Ten Pharmaceutical Company (Taipei, Taiwan) and Sheng Chang Pharmaceutical Company (Taipei, Taiwan).

2.3. Sample extraction

A sample of 100 mg of powdered plant material or commercial prescription was extracted with 1.0 mL of MeOH using sonication at room temperature for 30 min (three times). The combined extracts were evaporated to dryness. The residue was dissolved in 1.0 mL of a mixture of methanol-d_4 and benzene-d_6 (25:75) (contained 84.4 μg anthracene) and used for ^{1}H NMR measurement.

2.4. NMR analysis

^{1}H NMR spectra were recorded in a mixture of methanol-d_4 and benzene-d_6 (25:75) using a Varian UNITY plus 400 MHz spectrometer. For each sample, 100 scans were recorded with the following parameters: 0.187 Hz/point; spectra width, 14,400 Hz; pulse width, 4.0 μs; relaxation delay, 2 s. For quantitative analysis, peak area was used and the start and end point of the integration of each peak were selected manually.

2.5. Recovery

Pure berberine (**1**), palmatine (**2**) coptisine (**3**) and jatrorrhizine (**4**) were spiked into 100 mg of powered Rhizoma of *C. chinensis*. The recovery sample was prepared following the method described above. A blank recovery sample was prepared and analyzed for the comparison. Limit of detection (LOD) was evaluated at a signal-to-noise ratio of 3.

3. Results and discussion

In the ^{1}H NMR quantification of protoberberine alkaloids berberine, palmatine, coptisine and jatrorrhizine in Rhizoma Coptidis and commercial preparations, it would be desirable to quantify each individual alkaloid by means of the integral value of a specific proton signal. The protons H-13 and H-8 of these alkaloids resonating in a specific region of spectra as a singlet with little interference

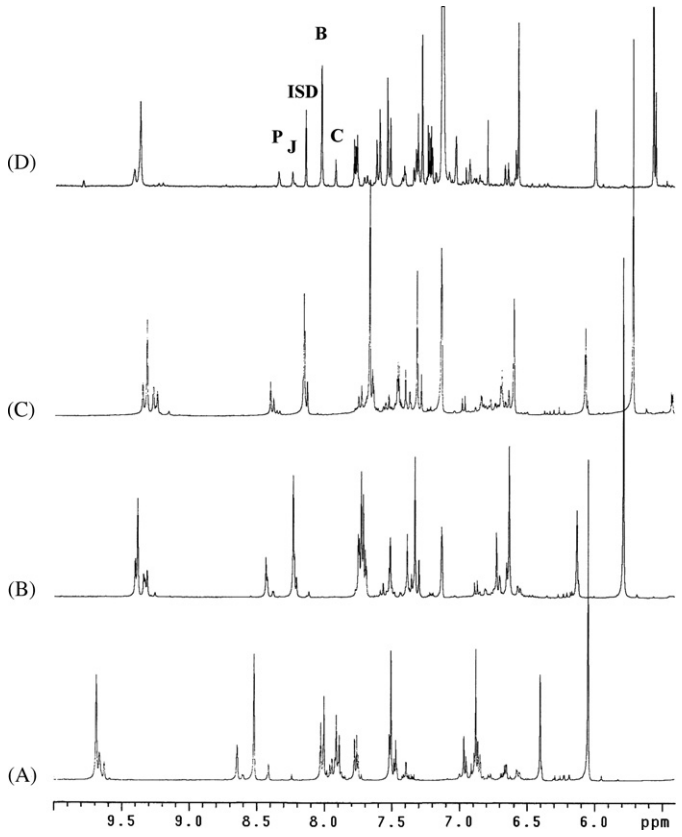

Fig. 2. ^{1}H NMR spectrum of the extract of *C. chinensis* in (A) methanol-d_4, (B) methanol-d_4–benzene-d_6 (80:20), (C) methanol-d_4–benzene-d_6 (55:45), and (D) methanol-d_4–benzene-d_6 (25:75) (contained the internal standard anthracene) in the range of δ 5.9–10.0 ppm. B: berberine, P: palmatine, C: coptisine, J: jatrorrhizine, and ISD: internal standard (anthracene).

were specific for these alkaloids and thus could be used for quantification. Because of these special characteristics, the H-13 signals were selected as the target signal for determining protoberberine alkaloids in the present study. However, these protoberberine alkaloids owned a quite similar skeleton and the ^{1}H NMR peaks of these constituents might interfere with each other. In previous studies, changing NMR solvents, controlling pH value, or addition of shift reagent were evaluated to solve this problem. In order to analyze these alkaloids efficiently and simultaneously, we selected a suitable solvent system for the detection.

To avoid the inference of phenolic hydroxyl signals and to more readily resolve the target signals, combination of methanol-d_4 with various solvents was evaluated. Methanol-d_4–benzene-d_6 combination was found to be the optimal NMR solvent system, as it showed a good separation of the H-13 peaks of berberine, palmatine, coptisine and jatrorrhizine. Accordingly, the effect of the percentage of benzene-d_6 on the separation of these signals was studied (Fig. 2). A 25:75 mixture of methanol-d_4 and benzene-d_6 was found to give well separation of target peaks originating from these alkaloids.

A suitable internal standard should be preferably a stable compound with a signal in a non-crowded region of the ^{1}H NMR spectrum. For this purpose, anthracene, with a signal at δ 8.14 ppm in the selected solvent system and the integral value maintaining constant within 48 h, has been chosen. In the case of ^{1}H NMR quantitative analysis, calibration curves were not needed for quantification of the compounds because integration of the peaks was always proportional to the amount of compounds and the same for all compounds in ^{1}H NMR.

Three sets of recovery samples were analyzed by the method as described above. The accuracy of the method was also checked by adding a known amount of berberine, plamatine, coptisine and jatrorrhizine (0.5, 1.0, and 2.0 mg) to the extract samples. The peak area corresponding to target constituent was found to increase proportionally with the added concentration of the standard and showed the average recovery rate between 96.2–102.8%, 95.9–105.2%, 97.2–99.1%, and 95.6–102.8% for berberine, palmatine, coptisine and jatrorrhizine, respectively (triplicate measurement). By using this method, the LOD for berberine was determined to be 0.03 mg/mL at a signal-to-noise ratio of 3.

By using the ^{1}H NMR method Rhizoma Coptidis materials including *C. chinensis*, *C. teeta* and *C. deltoidea*, and 23 samples of Rhizoma Coptidis commercial prescriptions from four different companies were analyzed for alkaloids: berberine, palmatine,

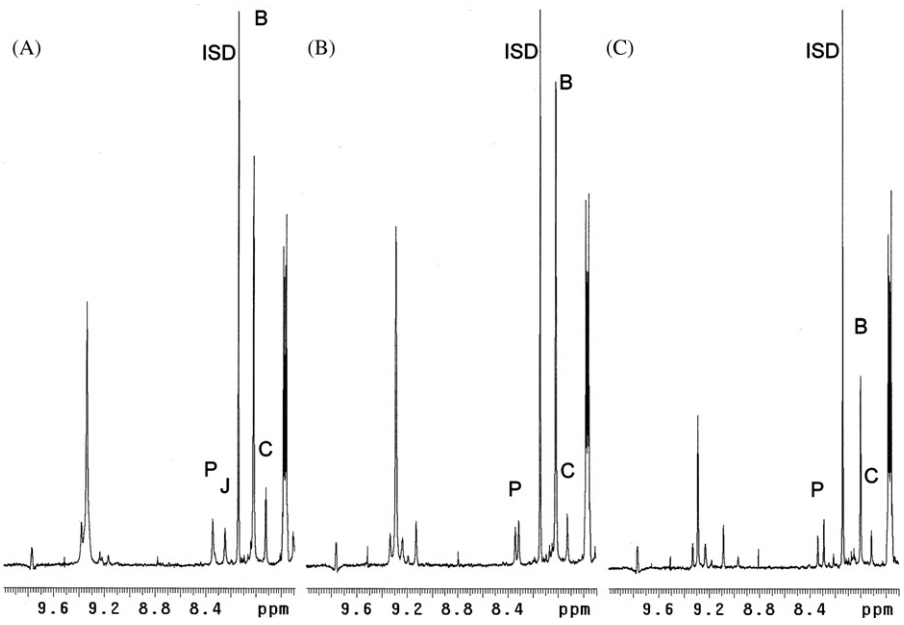

Fig. 3. The ^{1}H NMR spectra of Rhizoma Coptidis extracts: (A) *C. chinensis*, (B) *C. teeta*, and (C) *C. deltoidea* in the range of δ 7.7–10.0 ppm. B: berberine, P: palmatine, C: coptisine, J: jatrorrhizine, and ISD: internal standard (anthracene).

Table 1
The concentrations (mg/g) of berberine, palmatine, coptisine and jatrorrhizine in the Rhizoma of *C. chinensis*, *C. teeta* materials and Huang Lian commercial prescriptions[a].

Sample (company)		Palmatine	Jatrorrhizine	Berberine	Coptisine
C. chinensis		2.70 (0.6)[b]	1.31 (0.3)	12.97 (1.1)	2.27 (0.6)
C. teeta		0.66 (1.3)	–	11.71 (1.2)	1.98 (0.4)
C. deltoidea		1.93 (0.3)	–	8.31 (0.7)	2.35 (0.5)
Huang Lian Jie Du Tang	(A)	–	–	3.06 (1.1)	0.57 (0.4)
	(B)	0.80 (1.4)	0.47 (1.1)	2.75 (1.1)	0.89 (1.9)
	(C)	0.81 (0.3)	0.40 (1.2)	2.51 (1.7)	0.70 (0.9)
	(D)	0.80 (1.4)	0.53 (0.3)	2.25 (0.9)	0.66 (1.5)
Ge Geng Qin Lian Tang	(A)	0.47 (2.3)	0.18 (2.1)	1.55 (1.4)	0.49 (0.7)
	(B)	0.43 (2.0)	0.10 (2.4)	1.22 (2.0)	0.32 (1.3)
	(C)	0.73 (1.8)	0.05 (1.8)	1.70 (1.1)	0.53 (2.2)
	(D)	0.54 (1.4)	0.26 (2.2)	1.61 (0.1)	0.67 (1.8)
Qing Yin Li Ge Tang	(A)	–	–	0.40 (1.1)	–
	(B)	0.20 (2.2)	0.09 (2.2)	0.68 (1.4)	0.18 (0.6)
	(C)	0.29 (1.6)	0.12 (1.7)	0.96 (1.3)	0.28 (0.4)
	(D)	0.21 (1.1)	0.17 (0.9)	0.96 (2.2)	0.52 (1.1)
Chai Xian Tang	(A)	–	0.10 (2.4)	0.60 (0.3)	0.23 (0.4)
	(B)	0.51 (2.1)	0.36 (1.6)	1.78 (1.6)	0.70 (0.9)
	(C)	0.20 (2.2)	0.13 (2.3)	0.67 (0.8)	0.35 (1.4)
Huang Lian Tang	(A)	0.67 (0.6)	0.29 (1.3)	1.76 (0.5)	0.80 (1.7)
	(B)	0.53 (1.9)	0.25 (1.8)	1.90 (1.8)	0.58 (2.2)
Xiao Xian Xiong Tang	(B)	0.34 (0.7)	0.28 (2.4)	1.53 (0.7)	0.48 (1.5)
	(C)	0.41 (1.9)	0.21 (0.8)	1.15 (1.7)	0.32 (0.8)
Shu Gan Tang	(B)	0.38 (1.3)	0.35 (1.5)	1.56 (1.1)	0.57 (1.1)
	(C)	0.40 (2.2)	0.21 (2.1)	1.42 (0.6)	0.48 (1.7)
Pu Ji Xiao Du Yin	(C)	0.16 (1.7)	0.12 (1.7)	0.69 (1.5)	0.26 (0.3)
	(D)	0.40 (1.3)	0.26 (0.8)	1.69 (1.3)	0.56 (1.2)

[a] Recorded on mg/1 g of material.
[b] RSD%, all experiments were based on triplicate measurement.

coptisine and jatrorrhizine. Quantification of these four alkaloids by ^{1}H NMR was possible by means of the integral value of a well-separated specific proton signal of the compounds. For this purpose, the H-13 proton singlet of each alkaloid was selected as a target peak when it was quite well-separated from the others in the NMR spectrum obtained with the methanol-d_4 and benzene-d_6 (25:75) solvent system, and it could be integrated at this condition because it was in a region δ 7.8–8.4 where no interference from other signals occurred. The ^{1}H NMR spectra of Rhizoma Coptidis materials were shown in Fig. 3. In the ^{1}H NMR spectra of *C. chinesis*, berberine, palmatine, coptisine and jatrorrhizine were detected but berberine was determined to be the major constituent. The spectra of *C. teeta* contained the signals of berberine, palmatine and coptisine, but lack the signal of jatrorrhizine. A signal at δ 8.33 corresponding to an unknown protoberberine analogue with equal intensity as coptisine was observed. Besides, the signals of H-13 of berberine, palmatine, and coptisine were also observed in the spectrum of *C. deltoidea*, with an unknown protoberine analogue signal at δ 8.31. These spectra could be used as fingerprints for the identification of species used in the commercial Rhizoma Coptidis preparation.

The quantities of protoberberine alkaloids, berberine, palmatine, coptisine and jatrorrhizine in the three *Coptis* species and various Rhizoma Coptidis samples determined by this ^{1}H NMR method were shown in Table 1. The contents of protoberine alkaloids vary with plant materials. Berberine is the major alkaloid in these three species, and however, the berberine content in *C. deltoidea* was somewhat less than that in the other *Coptis* species. The various commercial Rhizoma Coptidis prescriptions produced by different companies were showed a quite different contents of these alkaloids (as shown in Table 1). These variations might be due to those different sources of materials purchased by different companies. For example, the alkaloids profile in *Huang Lian Jie Du Tang* produced by A company was very different with that of other three companies' products. It indicated that the material purchased in A company was totally different from that in other three companies. Besides, the alkaloids content in *Chai Xian Tang* produced by company B are about three times higher than that in A and C companies' products. This might be due to the abnormal addition the Rhizoma Coptidis to enhance the activity. Similar situations were appeared in the prescription *Pu Ji Xiao Do Yin*. Therefore, the developed ^{1}H NMR method satisfies the requirements for analysis of commercial *Coptis* products, and is reproducible for *Coptis* pharmaceutical preparations.

4. Conclusion

This NMR method is simple and rapid, specific, no reference compounds are needed, apart from the cheap internal standard, and an overall profile of the preparation can be obtained directly. By using this method, the contents of protoberberine alkaloids can be determined within much shorter time than by the conventional chromatographic or other analysis methods reported, and moreover without any derivatization. Overall profiles of several commercial Rhizoma Coptidis materials are successfully obtained by using this method. Thus, the described ^{1}H NMR method can be used as a rapid and simple method for the identification of *Coptis* species and quantification of protoberberine alkaloids in plant materials or commercial prescriptions.

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